

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

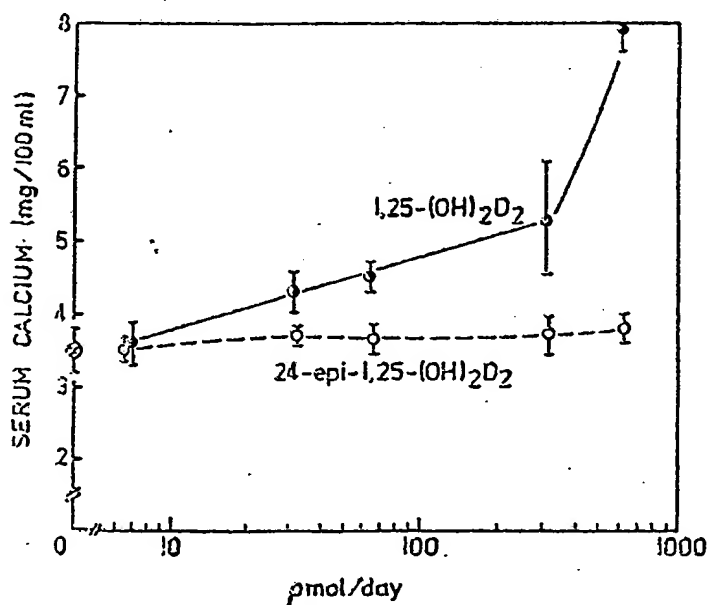
**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ³ : C07J 9/00; A61K 31/59	A1	(11) International Publication Number: WO 84/ 04527 (43) International Publication Date: 22 November 1984 (22.11.84)
(21) International Application Number: PCT/US84/00714 (22) International Filing Date: 9 May 1984 (09.05.84) (31) Priority Application Numbers: 492,863 607,327 (32) Priority Dates: 9 May 1983 (09.05.83) 4 May 1984 (04.05.84) (33) Priority Country: US (71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 North Walnut Street, Madison, WI 53705 (US). (72) Inventors: DeLUCA, Hector, F. ; 5130 Minocqua Crescent, Madison, WI 53705 (US). SCHNOES, Heinrich, K. ; 1806 Summit Avenue, Madison, WI 53705 (US). SICINSKI, Rafal, R. ; University of Warsaw, Department of Chemistry, 02-093 Warsaw, Pasteura 1 (PL). TANAKA, Yoko ; 613 Hilltop Drive, Madison, WI 53711 (US).	(74) Agent: BREMER, Howard, W.; 614 North Walnut Street, Madison, WI 53705 (US). (81) Designated States: AU, CH, DE, DK, JP, NL. Published With international search report.	

(54) Title: PROCESS FOR THE PREPARATION OF 1 α ,25-DIHYDROXYLATED VITAMIN D₂ AND RELATED COMPOUNDS



(57) Abstract

Novel derivatives of vitamin D₂ and more specifically 1 α ,25-dihydroxylated compounds of the vitamin D series. A process for the preparation of such derivatives is also provided as are certain intermediates in such process. The invention provides 1 α ,25-dihydroxyvitamin D₂ derivatives and acylates thereof, which find ready application as substitutes for vitamin D₃ or D₂ or various of the known vitamin metabolites of these vitamins in their various applications to the correction of disorders involving calcium metabolism and associated bone disease.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	KR	Republic of Korea
AU	Australia	LI	Liechtenstein
BE	Belgium	LK	Sri Lanka
BG	Bulgaria	LU	Luxembourg
BR	Brazil	MC	Monaco
CF	Central African Republic	MG	Madagascar
CG	Congo	MR	Mauritania
CH	Switzerland	MW	Malawi
CM	Cameroon	NL	Netherlands
DE	Germany, Federal Republic of	NO	Norway
DK	Denmark	RO	Romania
FI	Finland	SD	Sudan
FR	France	SE	Sweden
GA	Gabon	SN	Senegal
GB	United Kingdom	SU	Soviet Union
HU	Hungary	TD	Chad
JP	Japan	TG	Togo
KP	Democratic People's Republic of Korea	US	United States of America

Description

Process for the Preparation of $1\alpha,25$ -Dihydroxylated Vitamin D_2 and Related Compounds

Technical Field

This invention relates to the preparation of $1\alpha,25$ -dihydroxylated compounds of the vitamin D_2 series.

More specifically, this invention relates to the preparation of $1\alpha,25$ -dihydroxyvitamin D_2 and its (24R)-epimer, the corresponding 5,6-trans-isomers, and to certain C-25-alkyl or aryl analogs as well as the acyl derivatives of these compounds.

Background

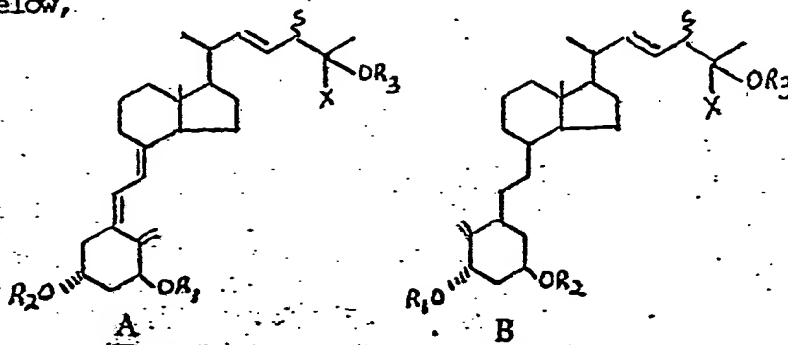
The importance of the hydroxylated forms of vitamin D as regulators of calcium and phosphate metabolism in animals and humans is by now well recognized through many disclosures in the patent and general literature, and as a consequence these hydroxyvitamin D derivatives are finding increasing clinical and veterinary use as medicaments for the treatment and cure of disorders of calcium metabolism and associated bone diseases. Vitamin D_3 is known to be hydroxylated in vivo to 25-hydroxyvitamin D_3 and then to $1\alpha,25$ -dihydroxyvitamin D_3 , the latter being generally accepted as the active hormonal form of vitamin D_3 . Similarly, the very potent vitamin D_2 metabolite, $1\alpha,25$ -dihydroxyvitamin D_2 ($1\alpha,25$ -(OH) $_2D_2$) is formed from vitamin D_2 via 25-hydroxyvitamin D_2 (25-OH- D_2). Both of these hydroxylated vitamin D_2 compounds have been isolated and identified (DeLuca et al, U.S. Patents 3,585,221; 3,880,894); being derived from vitamin D_2 , these metabolites are characterized by the (S)-stereochemistry at carbon 24.

Disclosure of Invention

A chemical process for preparing $1\alpha,25$ -dihydroxylated compounds of the vitamin D_2 series has now been developed. Specifically, this process provides a convenient means for



preparing compounds having the general structures A and B shown below,



wherein R_1 , R_2 , and R_3 are selected from the group consisting of hydrogen and acyl, and where X is an alkyl or aryl group. In these structures the asymmetric center at carbon 24 may have the (R) or (S) configuration.

Specific examples of compounds obtainable by the present process include $1\alpha,25$ -dihydroxyvitamin D_2 , the corresponding (24R)-epimer, $1\alpha,25$ -dihydroxy-24-epivitamin D_2 , the respective 5,6-trans-isomers, i.e. 5,6-trans- $1\alpha,25$ -dihydroxyvitamin D_2 , and 5,6-trans- $1\alpha,25$ -dihydroxy-24-epivitamin D_2 , as well as the C-25-alkyl or aryl homologs of these compounds, i.e. the compounds having the formulae shown above where X is ethyl, propyl, isopropyl or phenyl.

As used herein the term "acyl" signifies an aliphatic acyl group (alkanoyl group) of from 1 to 6 carbons, in all possible isomeric forms, e.g. formyl, acetyl, butyryl, isobutyryl, valeryl, etc., or an aromatic acyl group (aroyl group) such as benzoyl, or the methyl, halo, or nitro-substituted benzoyl groups, or an acyl group derived from a dicarboxylic acid having the general formulae $ROOC(CH_2)_nCO-$, or $ROOCH_2-O-CH_2CO-$, where n is an integer having the values of 0 to 4 inclusive, and R is hydrogen or an alkyl radical. Representative of such dicarboxylic acyl groups are oxalyl, malonyl, succinoyl, glutaryl, adipyl and diglycolyl. The term "alkyl" refers to a hydrocarbon group of 1 to 6 carbons in all isomeric forms, e.g. methyl, ethyl, propyl, isopropyl, butyl, isobutyl, etc. The term "aryl"

refers to an aromatic radical such as phenyl, benzyl, or the isomeric alkyl-substituted phenyl radicals.

An embodiment of the chemical process of this invention is depicted in appended Process Scheme I. In the following description of this process, numerals (e.g. 1, 2, 3, etc) designating specific products refer to the structures so numbered in Process Scheme I. A wavy line to the substituent (methyl) at C-24 indicates that this substituent may have either the R or S configuration.

A suitable starting material for the process of this invention is the vitamin D-ketal derivative of structure (1). It is generally convenient (e.g. in the case when both C-24-epimers of $1\alpha,25$ -dihydroxyvitamin D₂ compounds are desired) to use compound (1) as a mixture of the 24R and S epimers, separation of the individual 24R and S-epimers being accomplished at a later stage of the process. However, the pure 24S, or the pure 24R-epimer of (1) are equally suitable starting materials, whereby the former compound upon being processed through the indicated synthetic steps will provide the (24S)- $1\alpha,25$ -dihydroxy product, whereas the latter, treated analogously, will yield the corresponding (24R)- $1\alpha,25$ -dihydroxylated product.

Starting material (1) is converted to the desired 1α -hydroxylated form via cyclovitamin D derivatives (DeLuca et al., U.S. patents 4,195,027 and 4,260,549). Thus, treatment of compound (1) with toluenesulfonyl chloride in the conventional manner yields the corresponding C-3-tosylate (2), which is solvolized in an alcoholic medium to produce the novel 3,5-cyclovitamin D derivative (3). Solvolysis in methanol yields the cyclovitamin of structure (3) where Z=methyl, whereas the use of other alcohols, e.g. ethanol, 2-propanol, butanol, etc., in this reaction provides the analogous cyclovitamin D compounds (3), where Z is an alkyl group derived from the alcohol, e.g. ethyl, isopropyl, butyl, etc. Allylic oxidation of intermediate (3) with selenium



dioxide and a hydroperoxide yields the α -hydroxy-analog of structure (4). Subsequent acetylation of compound (4) provides the 1-acetate of structure (5, R_1 =acetyl). If desired, other 1-0-acylates (structure 5, where R_1 =acyl, e.g. the formate, propionate, butyrate, benzoate, etc.) are prepared by analogous conventional acylation reactions. The 1-0-acyl derivative is then subjected to acid-catalyzed solvolysis. When this solvolysis is conducted in a solvent medium containing water, there is obtained the 5,6-cis-vitamin D intermediate of structure (6, R_1 =acyl, R_2 =H) and the corresponding 5,6-trans-compound (structure 7, R_1 =acyl, R_2 =H) in a ratio of about 3-4:1. These 5,6-cis and 5,6-trans-isomers can be separated at this stage, e.g. by high performance liquid chromatography. If desired, the C-1-0-acyl group may be removed by base hydrolysis to obtain compounds (6) and (7) where R_1 and R_2 =H. Also if desired, these 1-0-monoacylates may be further acylated at the C-3-hydroxy groups, using conventional acylation conditions to obtain the corresponding 1,3-di-0-acylates of structure (6) or (7) where R_1 and R_2 , which may be the same or different, represent acyl groups. Alternatively, the hydroxy cyclovitamin of structure (4) can be subjected to acid-catalyzed solvolysis in a medium containing a low-molecular weight organic acid to obtain the 5,6-cis and trans compounds of structures (6) and (7) where R_1 =H and R_2 =acyl, where the acyl group is derived from the acid used in the solvolysis reaction.

The next step of the process comprises the removal of the ketal protecting group to produce the corresponding 25-ketone. This step is a critical one, since the ketal to ketone conversion must be accomplished without concomitant isomerization of the 22(23)-double bond to the conjugated 23(24)-position, which can occur under the acidic conditions required for ketal hydrolysis. Furthermore, conditions must be chosen so as to avoid elimination of the sensitive allylic C-1-oxygen function. The conversion is accomplished



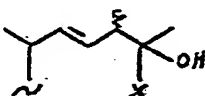
successfully by careful hydrolysis at moderate temperatures using organic acid catalysis. Thus, treatment of the 5,6-cis-compound (6) in aqueous alcohol with p-toluenesulfonic acid gives the corresponding ketone (8). To avoid undesired elimination of the C-1-oxygen function during this reaction, it is advantageous that the C-1-hydroxy group in compound (6) be protected (e.g. R_1 =acyl, R_2 =hydrogen or acyl).

Subsequent reaction of ketone (8) with a methyl-Grignard reagent then provides the desired 1 α ,25-dihydroxyvitamin D₂ compound of structure (9). If the starting material, compound (1), used in the above process, is a mixture of the two C-24-epimers, then compound (9) will be obtained as a mixture of the 24S and R-epimers (9a and 9b, respectively). Separation of this epimer mixture can be achieved by chromatographic methods, to obtain 1 α ,25-dihydroxyvitamin D₂ (structure 9a, 24S-stereochemistry) and its 24R-epimer, 1 α ,25-dihydroxy-24-epivitamin D₂, of structure 9b, both in pure form. Such separation of epimers is, of course, not necessary if the compounds are intended to be used as a mixture.

The 5,6-trans-25-ketal-intermediate of structure (7), subjected to ketal hydrolysis in an analogous manner, provides the 5,6-trans ketone intermediate of structure (10), which via a Grignard reaction with methyl magnesium bromide or analogous reagent gives the 5,6-trans-1 α ,25-dihydroxyvitamin D₂ compounds of structure (11), as the 24S or 24R-epimer, or as a mixture of both epimers depending on the nature of the starting material (1) used in the process. If obtained as an epimeric mixture, the epimers can be separated by chromatography, to obtain 5,6-trans-1 α ,25-dihydroxyvitamin D₂ (11a) and its 24R-epimer, 5,6-trans-1 α ,25-dihydroxy-24-epivitamin D₂, of structure (11b). These reaction steps utilizing the 5,6-trans-intermediate are conducted in a manner entirely analogous to those applicable to the 5,6-cis-compounds described above.



The novel side chain ketones of structures (8) or (10) are most useful and versatile intermediates in that they can be used to prepare a variety of $1\alpha,25$ -dihydroxyvitamin D_2 -side chain analogs. Specifically, these keto-intermediates can serve for the preparation of 5,6-cis- or 5,6-trans- $1\alpha,25$ -dihydroxyvitamin D_2 analogs having the general side chain formula shown below,



where X is an alkyl or aryl group.

For example, treatment of ketone (8) with ethyl magnesium bromide gives the corresponding hydroxyvitamin D_2 analog having the side chain structure shown above wherein X is ethyl group. Likewise, treatment of (8) with isopropyl magnesium bromide or phenyl magnesium bromide gives the side chain analogs where X is isopropyl or phenyl, respectively. Analogous treatment of the 5,6-trans-25-ketone intermediate of structure (10) with alkyl or aryl-Grignard reagents gives the 5,6-trans-vitamin D_2 analog having the side chain above where X is the alkyl or aryl radical introduced by the Grignard reagent employed.

It is also evident that the reaction of the keto-intermediates (8) or (10) with an isotopically-labeled Grignard reagent (e.g. C^3H_3MgBr , $^{14}CH_3MgBr$, C^2H_3MgBr , etc.) provides a convenient means for preparing $1\alpha,25$ -dihydroxyvitamin D_2 or its trans isomer, and the corresponding C-24-epimers, in isotopically-labeled form, i.e. as the compounds having the side chain shown above, wherein X is C^3H_3 , $^{14}CH_3$, C^2H_3 , $^{13}CH_3$, or any other isotopically-labeled alkyl or aryl group selected.

The above alkyl or aryl homologues of the 5,6-cis or trans- $1\alpha,25$ -dihydroxyvitamin D_2 are useful substitutes of the parent compounds in situations where a greater degree of lipophilicity is desired, whereas the isotopically labeled



compounds referred to above, find use as reagent in analytical applications.

Further, although for therapeutic applications, the free hydroxy compounds represented by structures A and B above (where R_1 , R_2 and $R_3=H$) are generally used, for some such applications, the corresponding hydroxy-protected derivatives may be useful or preferred. Such hydroxy-protected derivatives are for example the acylated compounds represented by general formulae A and B above, wherein one or more of R_1 , R_2 , and R_3 represents an acyl group.

Such acyl derivatives are conveniently prepared from the free hydroxy compounds by conventional acylation procedures, i.e. treatment of any of the hydroxyvitamin D_2 products with an acyl halide, or acid anhydride in a suitable solvent such as pyridine, or an alkyl-pyridine. By appropriate selection of reaction time, acylating agent, temperature and solvent, as is well-known in the art, the partially or fully acylated derivatives represented by structures A or B above are obtained. For example, treatment of 1 α ,25-dihydroxyvitamin D_2 (9a) in pyridine solvent with acetic anhydride at room temperature gives the 1,3-diacetate, while the same reaction conducted at elevated temperature yields the corresponding 1,3,25-triacetate. The 1,3-diacetate can be further acylated at C-25 with a different acyl group; e.g. by treatment with benzoyl chloride or succinic anhydride there is obtained the 1,3-diacetyl-25-benzoyl-, or 1,3-diacetyl-25-succinoyl-derivative, respectively. A 1,3,25-triacyl derivative can be selectively hydrolyzed in mild base to provide the 1,3-dihydroxy-25-O-acyl compound, the free hydroxy groups of which can be reacylated, if desired, with different acyl groups. Likewise, a 1,3-diacyl derivative can be subjected to partial acyl hydrolysis to obtain the 1-O-acyl and the 3-O-acyl compounds, which in turn can be reacylated with different acyl groups. Like treatment of any of the other hydroxyvitamin D_2 products (e.g. 9b, 11a/b, or their



corresponding 25-alkyl or aryl analogs) provides the corresponding desired acyl derivatives represented by structures A or B, where any or all of R_1 , R_2 , and R_3 are acyl.

Like the previously known vitamin D_2 metabolite, 1α , 25-dihydroxyvitamin D_2 (9a), the novel compounds of this invention exhibit pronounced vitamin D-like activity, and thus represent desirable substitutes for the known vitamin D_2 or D_3 metabolites in many therapeutic or veterinary applications. Particularly preferred in this regard are the products of structure 9b and 11a and 11b, or their acylated derivatives. The novel compounds may be used for correcting or improving a variety of calcium and phosphate imbalance conditions resulting from a variety of diseases, such as vitamin D-resistant rickets, osteomalacia, hypoparathyroidism, osteodystrophy, pseudohypoparathyroidism, osteoporosis, Paget's disease, and similar bone and mineral-related disease states known to the medical practice. The compounds can also be used for the treatment of mineral imbalance conditions in animals, for example, the milk fever condition, poultry leg weakness, or for improving egg shell quality of fowl. Their use in the treatment of osteoporosis is particularly noteworthy.

It is well known that females at the time of menopause suffer a marked loss of bone mass giving rise ultimately to osteopenia, which in turn gives rise to spontaneous crush fractures of the vertebrae and fractures of the long bones. This disease is generally known as postmenopausal osteoporosis and presents a major medical problem, both in the United States and most other countries where the life-span of females reaches ages of at least 60 and 70 years. Generally the disease which is often accompanied by bone pain and decreased physical activity, is diagnosed by one or two vertebral crush fractures with X-ray evidence of diminished bone mass. It is known that this disease is accompanied by diminished ability to absorb calcium, decreased levels of sex hormones,



especially estrogen and androgen, and a negative calcium balance.

Methods for treating the disease have varied considerably. For example, calcium supplementation by itself has not been successful in preventing or curing the disease and the injection of sex hormones, especially estrogen, which has been reported to be effective in preventing the rapid loss of bone mass experienced in postmenopausal women, has been complicated by the fear of its possible carcinogenicity. Other treatments, for which variable results have again been reported, have included a combination of vitamin D in large doses, calcium and fluoride. The primary problem with this approach is that fluoride induces structurally unsound bone, called woven bone, and in addition, produces a number of side effects such as increased incidence of fractures and gastrointestinal reaction to the large amounts of fluoride administered.

Similar symptoms characterize senile osteoporosis and steroid-induced osteoporosis, the latter being a recognized result of long term glucocorticoid (cortico-steroid) therapy for certain disease states.

While various metabolites of vitamin D₃ increase calcium absorption and retention within the body of mammals displaying evidence of or having a physiological tendency toward loss of bone mass they are also characterized by the complementary vitamin D-like characteristic of mobilizing the calcium in bone in response to physiological needs. It has now been found that the epi compounds of this invention, especially 24-epi-1 α ,25-dihydroxyvitamin D₂ (24-epi-1,25-(OH)₂D₂), are eminently suitable for the prevention or treatment of physiological disorders in mammals which are characterized by the loss of bone mass because, although they express some of the recognized vitamin D-like characteristics affecting calcium metabolism, such as, increasing intestinal calcium transport, and effecting bone mineralization, they do not



increase serum calcium levels, even at high dosages. This observed characteristic evinces that the compounds upon administration, do not mobilize bone. This fact, along with the ability of the compounds upon administration to mineralize bone, indicates that they are ideal compounds for the prevention or treatment of prevalent calcium disorders which are evidenced by loss of bone mass, for example postmenopausal osteoporosis, senile osteoporosis and steroid-induced osteoporosis. It will be evident that the compounds will find ready application for the prevention or treatment of other disease states in which the loss of bone mass is an indication such as in the treatment of patients undergoing renal dialysis where loss of bone mass as a consequence of the dialysis is encountered.

The following Examples will serve to illustrate the characteristics of 24-epi-1,25-(OH)₂D₂ which contribute to its eminent suitability for the prevention or treatment of disease states that evince bone mass loss.

Example 1

Weanling male rats were placed on the vitamin D deficient diet described by Suda et al., Journal of Nutrition 100, 1049-1052 (1970), modified to contain .02% calcium and .3% phosphorus. After two weeks on this diet, the animals were given either 1,25-dihydroxyvitamin D₂, or 24-epi-1,25-dihydroxyvitamin D₂ daily by subcutaneous injection in 0.1 ml of 5% ethanol in propanediol. Twelve hours after the last dose, the animals were killed and the blood calcium and intestinal calcium transport measured. The results of these measurements for the indicated levels of the compounds administered are shown in Figures 1 and 2. The intestinal calcium transport measurements shown in Figure 2 were performed by the method of Martin and DeLuca, American Journal of Physiology 216, 1351-1359 (1969).



Example 2

Weanling male rats were placed on a high calcium (1.2% calcium) and low phosphorus (.1% phosphorus) diet described by Suda et al (supra). The rats were fed this diet for a period of three weeks at which time they were separated into two groups. One group was given $1,25(\text{OH})_2\text{D}_2$ while the other group was given 24-epi- $1,25(\text{OH})_2\text{D}_2$, both in 0.1 ml of 5% ethanol in propane diol subcutaneously at the dosage levels of the compounds shown by the data points in Figure 3. These doses were continued daily for a period of seven days, at which time the animals were killed and serum inorganic phosphorus determined. Results are shown in Figure 3.

Bone ash was determined by removing the femurs from rats. The femurs were dissected free of adhering connective tissue, extracted for 24 hours in absolute ethanol, and 24 hours in diethyl ether, using a Soxhlet extractor. The bones are ashed at 600°F for 24 hours. The ash weight was determined by weighing to constant weight. Results are shown in Figure 4.

The results of the two studies described in Examples 1 and 2, above, illustrate that 24-epi- $1,25(\text{OH})_2\text{D}_2$ is approximately equal in potency to $1\alpha,25$ -dihydroxyvitamin D_3 ($1,25(\text{OH})_2\text{D}_3$) in causing the mineralization of bone and in stimulating intestinal calcium transport. In short, there is no significant difference between the two groups in Figure 2 and Figure 4. On the other hand, the elevation of serum inorganic phosphorus which results from mobilization of bone in the case of the low phosphorus diet is very markedly affected by $1,25(\text{OH})_2\text{D}_2$, but hardly stimulated by 24-epi- $1,25(\text{OH})_2\text{D}_2$. Similarly, in the mobilization of calcium from bone, as indicated by the serum calcium levels (Figure 1) even at the extremely high dose level of about 750 pmoles/day, the 24-epi compound had no effect, while the mobilization effect is evident at much lower doses of $1,25$ -dihydroxyvitamin D_2 . Since the rise in serum calcium of rats on a low calcium diet measures the ability to mobilize bone, and since the elevation



of blood phosphorus of animals on a low phosphorus diet also measures bone mobilization, these results show that 24-epi-1,25-(OH)₂D₂ provides an unexpected property, namely that it is of minimal effectiveness in mobilizing bone calcium, while being fully able to stimulate intestinal calcium transport and the mineralization of new bone, properties which make this compound highly suitable for the treatment of disease states that evince bone loss.

The unique characteristics of 24-epi-1,25-(OH)₂D₂, as set forth above, offer the rare opportunity to control the various vitamin D-responsive processes (intestinal calcium absorption, bone mineral mobilization, and bone mineralization) in a manner and to a degree heretofore not feasible. This possibility arises from the fact that the 24-epi compound of this invention may be administered to the mammal either alone (with suitable and acceptable excipients) or in combination with other vitamin D-derivatives which exhibit the full spectrum of D-like activity. By such measures, it is possible therefore to combine (to whatever degree desired) the specificity of action of the 24-epi-analog with the generality of action of other vitamin D metabolites or analogs.

Administration of 24-epi-1,25-(OH)₂D₂ alone will, as shown above, stimulate intestinal calcium transport and bone mineralization with no or minimal bone mineral mobilization, but the latter activity can be induced by co-administration of one or more of the known vitamin D derivatives (e.g., 1,25-(OH)₂D₃, 1 α ,25-(OH)₂D₂, 1 α -OH-D₃, and related analogs). By adjusting the relative amounts of compounds administered, a degree of control over the relative magnitudes of the intestinal calcium absorption vs. bone mineral mobilization processes can be exercised in a manner not possible with the heretofore known vitamin D derivatives. Co-administration of the 24-epi compound and other vitamin D compounds with bone mobilizing activity can be particularly advantageous in situation where some degree of bone mobilization is desired.



For example, it is believed that in certain circumstances, bone must first be mobilized before new bone can be laid down. In such situations treatment with vitamin D or a vitamin D derivative which will induce bone mobilization, e.g. 1α -hydroxyvitamin D_3 or $-D_2$, $1\alpha,25$ -dihydroxyvitamin D_3 or $-D_2$, 25 -hydroxyvitamin D_3 or $-D_2$, $24,24$ -difluoro- 25 -hydroxyvitamin D_3 , $24,24$ -difluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , 24 -fluoro- 25 -hydroxyvitamin D_3 , 24 -fluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , 2β -fluoro- 1α -hydroxyvitamin D_3 , 2β -fluoro- 25 -hydroxyvitamin D_3 , 2β -fluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , $26,26,26,27,27,27$ -hexafluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , $26,26,26,27,27,27$ -hexafluoro- 25 -hydroxyvitamin D_3 , $24,25$ -dihydroxyvitamin D_3 , $1\alpha,24,25$ -trihydroxyvitamin D_3 , $25,26$ -dihydroxyvitamin D_3 , $1\alpha,25,26$ -trihydroxyvitamin D_3 , in combination with 24 -epi- $1,25(OH)_2D_2$ will, by adjustment of the proportions of the 24 -epi compound and the bone-mobilizing vitamin D compound in the treatment regimen permit the rate of mineralization of bone to be adjusted to achieve the desired medical and physiological ends. Suitable and effective mixtures are for example, the combination of $1\alpha,25$ -dihydroxyvitamin D_2 and $1\alpha,25$ -dihydroxy- 24 -epivitamin D_3 (9a and 9b), or mixtures of the corresponding 5,6-trans-compounds (11a and 11b), or any other combination of these four products as the free hydroxy compounds or as their acylated forms.

The compounds of this invention or combinations thereof with other vitamin D derivatives or other therapeutic agents, can be readily administered as sterile parenteral solutions by injection or intravenously, or by alimentary canal in the form of oral dosages, or trans-dermally, or by suppository. Advantageously the compounds are administered in dosage amounts of from 0.1 to 100 micrograms per day. In relation to osteoporosis, doses from about 0.5 to about 25 micrograms per day are generally effective. The compounds can be administered either alone or in combination with other vitamin D derivatives, the proportions of each of the compounds in the combination being



dependent upon the particular disease state being addressed and the degree of bone mineralization and/or bone mobilization desired. In the treatment of osteoporosis where the preferred compound is 24-epi- $1\alpha,25-(OH)_2D_2$ the actual amount of the 24-epi-compound used is not critical. In all cases, sufficient of the compound should be used to induce bone mineralization. Amounts in excess of about 25 micrograms per day of the 24-epi-compound or the combination of that compound with bone mobilization-inducing vitamin D derivatives, are generally unnecessary to achieve the desired results and may not be economically sound practice. In practice, higher doses of the compounds are used where therapeutic treatment of a disease state is the desired end while the lower doses are generally used for prophylactic purposes, it being understood that the specific dosage administered in any given case will be adjusted in accordance with the specific compounds being administered, the disease to be treated, the condition of the subject and the other relevant medical facts that may modify the activity of the drug or the response of the subject, as is well known by those skilled in the art.

Dosage forms of the compounds can be prepared by combining them with non-toxic pharmaceutically acceptable carriers as is well known in the art. Such carriers may be either solid or liquid such as, for example, corn starch, lactose, sucrose, peanut oil, olive oil, sesame oil and propylene glycol. If a solid carrier is used the dosage form of the compounds may be tablets, capsules, powders, troches or lozenges. If a liquid carrier is used, soft gelatin capsules, or syrup or liquid suspensions, emulsions or solutions may be the dosage form. The dosage forms may also contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, etc. They may also contain other therapeutically valuable substances such as other vitamins,



salts, sugars, proteins, hormones or other medicinal compounds.

The process of the present invention is more particularly described by Examples 3 through 9 which follow. In these examples the designation of specific products by Arabic numerals (e.g. compounds 1, 2, 3, etc.) refer to the structures so numbered in Process Scheme I.

Example 3

1 α -hydroxy-3,5-cyclovitamin D (4, Z=methyl).

A solution of compound (1) (50 mg) (as a mixture of the 24 R and S epimers) in dry pyridine (300 μ l) is treated with 50 mg of p-toluenesulfonyl chloride at 4°C for 30 h. The mixture is poured over ice/sat. NaHCO₃ with stirring and the product is extracted with benzene. The combined organic phases are washed with aqueous NaHCO₃, H₂O, aqueous CuSO₄ and water, dried over MgSO₄ and evaporated.

The crude 3-tosyl derivative (2) is directly solvolyzed in anhydrous methanol (10 ml) and NaHCO₃ (150 mg) by heating at 55°C for 8.5 h with stirring. The reaction mixture is then cooled to room temperature and concentrated to ~2 ml under vacuo. Benzene (80 ml) is then added and organic layer is washed with water, dried and evaporated. The resulting cyclovitamin (3, Z=methyl) can be used in the subsequent oxidation without further purification.

The crude product (3) in CH₂Cl₂ (4.5 ml) is added to an ice-cooled solution at SeO₂ (5.05 mg) and t-BuOOH (16.5 μ l) in CH₂Cl₂ (8 ml) containing anhydrous pyridine (50 μ l). After being stirred for 15 min at 0°C, the reaction mixture is allowed to warm to room temperature. After an additional 30 min, the mixture is transferred to a separatory funnel and shaken with 10% NaOH (30 ml). Ether (150 ml) is added and the separated organic phase is washed with 10% NaOH, water, dried and evaporated. The oily residue is purified on silica gel thin layer plates (20 x 20 cm plates, AcOEt/hexane 4:6) to



yield 20 mg of α -hydroxy derivative (4, Z=methyl): mass spectrum, m/e : 470 (M^+ , 5), 438 (20), 87 (100); NMR ($CDCl_3$) δ 0.53 (3H, s, 18- H_3), 0.63 (1H, m, 3-H), 4.19 (1H, d, J=9.5 Hz, 6-H), 4.2 (1H, m, 1-H), 4.95 (1H, d, J=9.5 Hz, 7-H), 5.17 and 5.25 (2H, each m, 19- H_2), 5.35 (2H, m, 22-H and 23-H).

Example 4

Acetylation of compound (4).

A solution of cyclovitamin (4, Z=methyl) (18 mg) in pyridine (1 ml) and acetic anhydride (0.33 ml) is heated at 55°C for 2 h. The mixture is poured into ice-cooled sat. $NaHCO_3$ and extracted with benzene and ether. The combined organic extracts are washed with water, saturated $CuSO_4$ and aqueous $NaHCO_3$ solutions, dried and evaporated to give 1-acetoxy derivative (5, Z=methyl, acyl=acetyl) (19 mg): mass spectrum, m/e : 512 (M^+ , 5), 420 (5), 87 (100); NMR ($CDCl_3$) δ 0.53 (3H, s, 18- H_3), 4.18 (1H, d, J=9.5 Hz, 6-H), 4.97 (2H, m, 7-H and 19-H), 5.24 (2H, m, 1-H and 19-H), 5.35 (2H, m, 22-H and 23-H).

Example 5

Solvolysis of α -acetoxy-3,5-cyclovitamin (5)

(R_1 =acetyl).

A solution of cyclovitamin (5) (4.5 mg) in 3:1 mixture of dioxane/ H_2O (1.5 ml) is heated at 55°C. p-Toluenesulfonic acid (1 mg in 20 μ l of H_2O) is then added and heating is continued for 15 min. The mixture is poured into saturated $NaHCO_3$ /ice, and extracted with benzene and ether. The organic phases are washed with $NaHCO_3$ and water and dried over $MgSO_4$. Evaporation of solvents gives a residue containing compounds (6) (where R_1 =acetyl and R_2 =H) and (7) (where R_1 =acetyl and R_2 =H) which are separated by chromatography on HPLC (6.2 mm x 25 cm Zorbax-Sil) using 2% of 2-propanol in hexane as an eluent. If necessary, the products are further purified by rechromatography.

Example 6

Ketal hydrolysis in compound (6) to obtain ketone (8).



To the solution of ketal (6, R_1 =acetyl, R_2 =H) (1.35 mg) in ethanol (1.5 ml), p-toluenesulfonic acid (0.34 mg in 45 μ l of H_2O) is added and the mixture is heated under reflux for 30 min. The reaction mixture is poured into diluted $NaHCO_3$, and extracted with benzene and ether. The combined organic extracts are washed with water, dried over $MgSO_4$ and evaporated. High-pressure liquid chromatography of the crude mixture (4% 2-propanol/hexane, 6.2 mm x 25 cm Zorbax-Sil) affords some unreacted ketal (6) (0.12 mg, collected at 48 ml) and desired ketone (8, R_1 =acetyl, R_2 =H) (0.36 mg, collected at 52 ml), characterized by the following data: mass spectrum, m/e : 454 (M^+ , 9), 394 (17), 376 (10), 134 (23), 43 (100); NMR ($CDCl_3$) δ 0.53 (3H, s, 18- H_3), 1.03 (3H, d, $J=6.5$ Hz, 21- H_3), 1.13 (3H, d, $J=7.0$ Hz, 28- H_3), 2.03 (3H, s, CH_3COO), 2.12 (3H, s, CH_3CO), 4.19 (1H, m, 3-H), 5.03 (1H, m, 19-H), 5.33 (3H, broad m, 19-H, 22-H and 23-H), 5.49 (1H, m, 1-H), 5.93 (1H, d, $J=11$ Hz, 7-H), 6.37 (1H, d, $J=11$ Hz, 6-H); UV (EtOH) λ_{max} 266 nm, 250 nm, λ_{min} 225 nm.

Example 7

Reaction of ketone (8) with methylmagnesium bromide to obtain products (9a) and (9b).

Ketone (8, R_1 =acetyl, R_2 =H) in anhydrous ether is treated with the excess of CH_3MgBr (2.85 M solution in ether). The reaction mixture is stirred at room temperature for 30 min, then quenched with aq. NH_4Cl , extracted with benzene, ether and CH_2Cl_2 . The organic phases are washed with dilute $NaHCO_3$, dried over $MgSO_4$ and evaporated. The mixture of (9a) and (9b) thus obtained is separated by high performance liquid chromatography (6% 2-propanol/hexane, 4.6 mm x 25 cm Zorbax-Sil), to obtain, in order of elution, pure epimers (9a) and (9b). $1\alpha,25$ -dihydroxyvitamin D_2 (9a): UV (EtOH) λ_{max} 265.5 nm, λ_{min} 227.5 nm; mass spectrum, m/e 428 (M^+ , 6), 410 (4), 352 (4), 287 (6), 269 (10), 251 (10), 152 (42), 134 (100), 59 (99); NMR ($CDCl_3$) δ 0.56 (3H, s, 18- H_3), 1.01 (3H, d, $J=6.5$ Hz, 28- H_3), 1.04 (3H, d, $J=6.5$ Hz, 21- H_3), 1.14 and



1.18 (6H, each s, 26-H₃ and 27-H₃), 4.24 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.01 (1H, m, 19-H), ~5.34 (3H, broad m, 19-H, 22-H and 23-H), 6.02 (1H, d, J=11 Hz, 7-H), 6.39 (1H, d, J=11 Hz, 6-H).

1 α ,25-dihydroxy-24-epivitamin D₂ (9b): UV (EtOH) λ_{\max} 265.5 nm, λ_{\min} 227.5 nm; mass spectrum, m/e 428 (M⁺, 13), 410 (9), 352 (7), 287 (11), 269 (15), 251 (13), 152 (52), 134 (100), 59 (97).

Example 8

Conversion of compound (7) to 5,6-trans-1 α ,25-dihydroxy-vitamin D₂ compounds (11a) and (11b).

Hydrolysis of ketal-intermediate (7, R₁=acetyl, R₂=H) using the conditions described in Example 4 provides the corresponding 5,6-trans-25-ketone of structure (10, R₁=acetyl, R₂=H), and subsequent reaction of this ketone with methyl magnesium bromide, using conditions analogous to those of Example 5, gives a mixture of epimers (11a) and (11b) which are separated by high performance liquid chromatography (HPLC) to obtain in pure form 1 α ,25-dihydroxy-5,6-trans-vitamin D₂ (11a) and 1 α ,25-dihydroxy-5,6-trans-24-epivitamin D₂ (11b). If required, structure assignment can be confirmed by isomerization to the respective 5,6 cis compounds (9a, 9b) according to known procedures.

5,6-trans-1 α ,25-dihydroxyvitamin D₂ (11a): UV (EtOH) λ_{\max} 273.5 nm, λ_{\min} 230 nm; mass spectrum, m/e 428 (M⁺, 8), 410 (3), 287 (3), 269 (7), 251 (7), 152 (34), 134 (100), 59 (78).

5,6-trans-1 α ,25-dihydroxy-24-epivitamin D₂ (11b): UV (EtOH) λ_{\max} 273.5 nm, λ_{\min} 230 nm; mass spectrum, m/e 428 (M⁺, 10), 410 (4), 352 (4), 287 (5), 269 (9), 251 (8), 152 (37), 134 (100), 59 (82).

Example 9

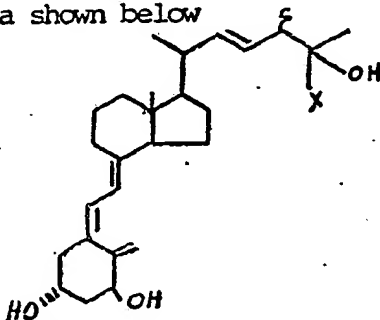
Preparation of alkyl and aryl analogs of 1 α ,25-dihydroxy-vitamin D₂ compounds.

By reaction of ketone intermediate (8) (R₁=acetyl, R₂=H) with, respectively,



- (a) ethyl magnesium bromide
- (b) propyl magnesium bromide
- (c) isopropyl magnesium bromide
- (d) butyl magnesium bromide
- (e) phenyl magnesium bromide

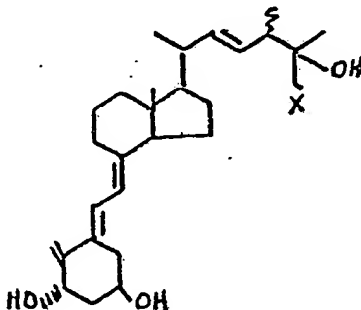
using conditions analogous to those described in Example 7, there are obtained the corresponding hydroxyvitamin D₂ products having the formula shown below



wherein X is, respectively

- (a) ethyl
- (b) propyl
- (c) isopropyl
- (d) butyl
- (e) phenyl

By like treatment of 5,6-trans-ketone intermediate (10) (R_1 =acetyl, R_2 =H) with the above listed Grignard reagents, there are obtained the corresponding 5,6-trans-hydroxyvitamin D₂ products, having the formula shown below



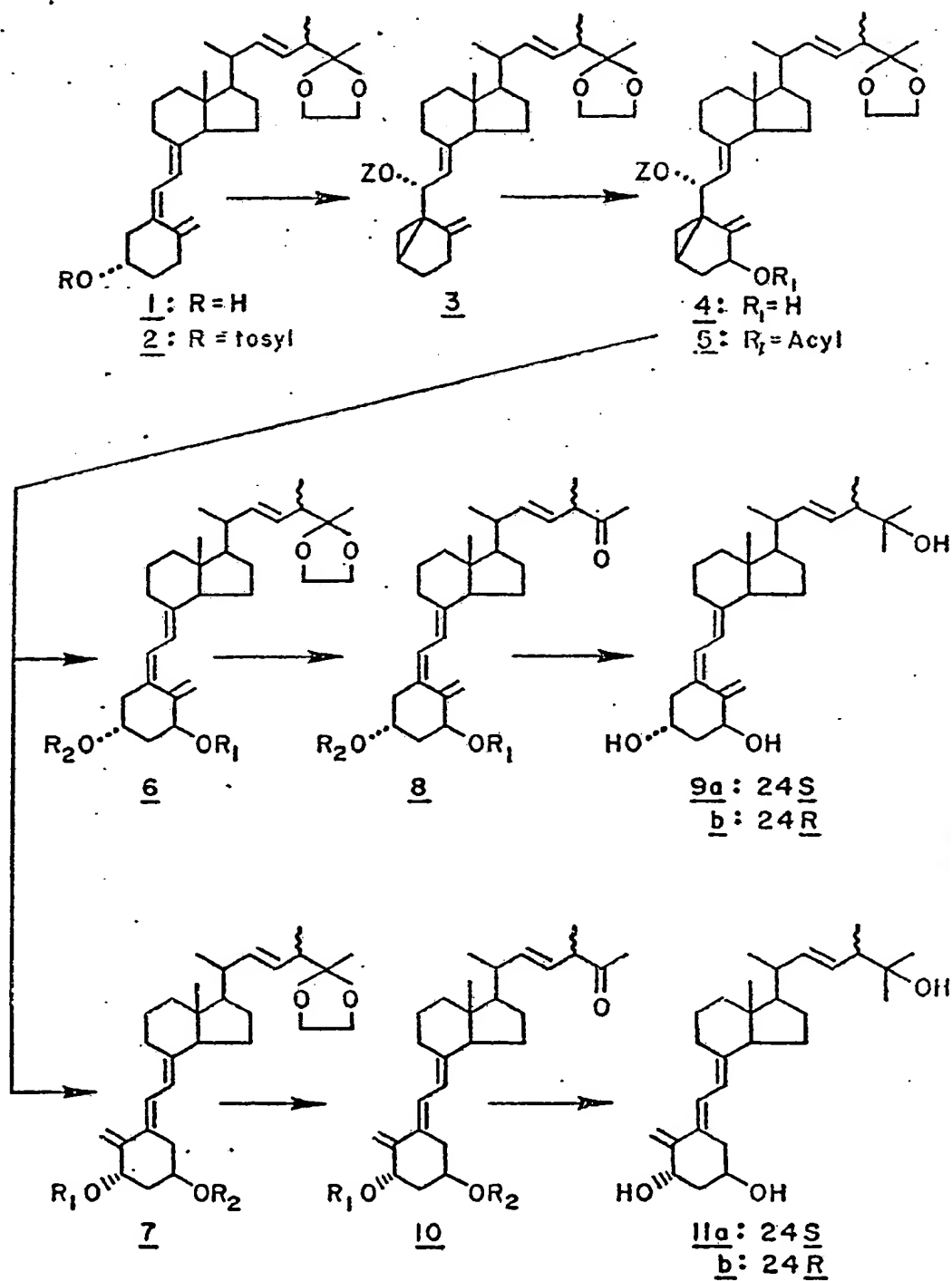
wherein X is, respectively

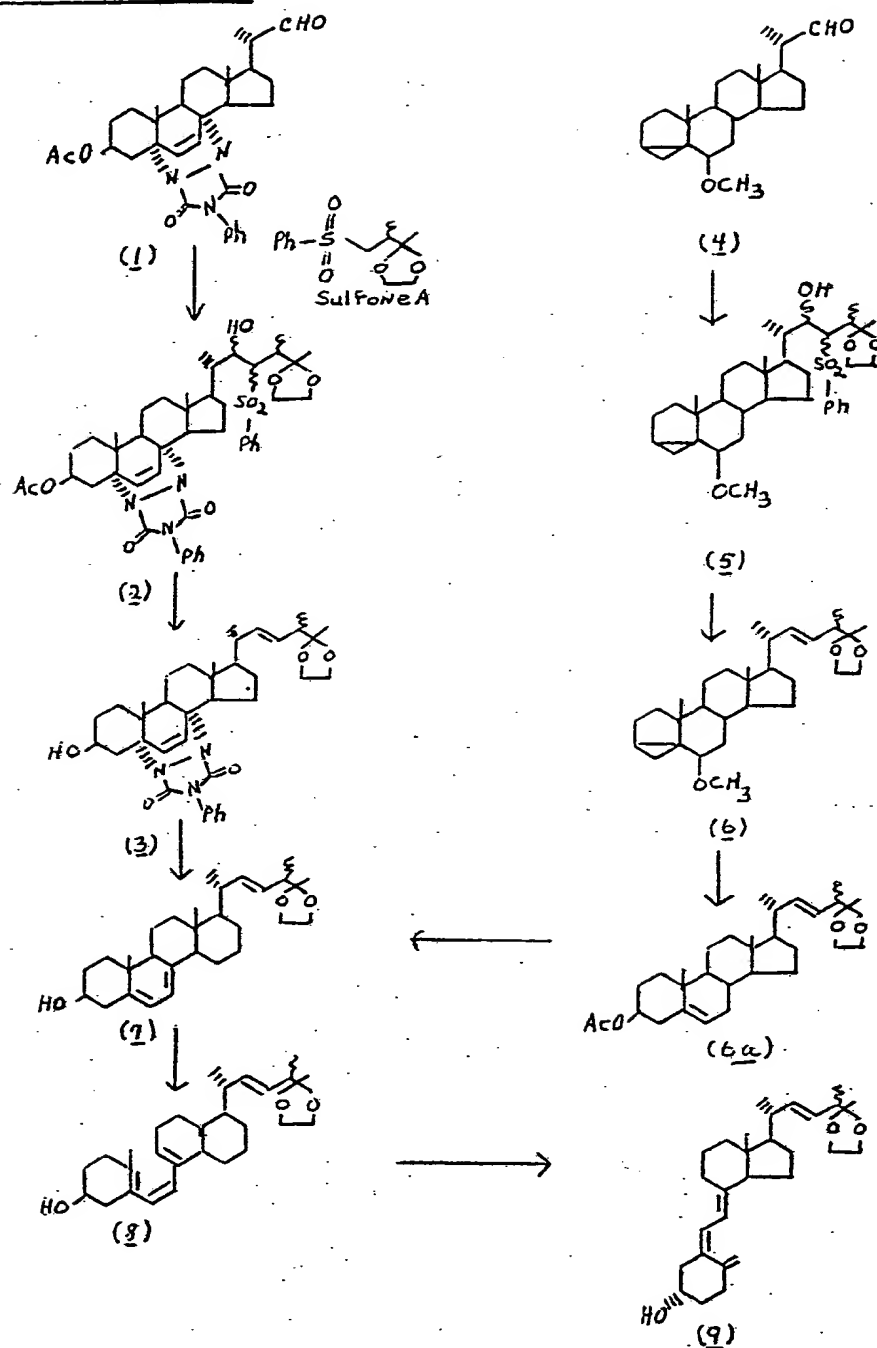
- (a) ethyl
- (b) propyl
- (c) isopropyl
- (d) butyl
- (e) phenyl

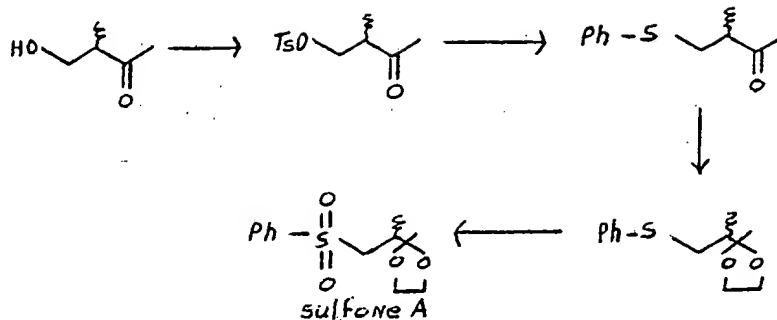
A suitable starting material for the process of this invention is the vitamin D-ketal derivative of structure (1) which can be obtained following Process Schemes II and III as described in British Specification No. 2,127,023 or United States Letters Patent No. 4,448,721. It is generally convenient (e.g. when both C-24-epimers are desired) to use compound (1) as a mixture of 24R and 24S epimers, separation of the individual 24R and 24S epimers being accomplished later. However, pure 24S-, or pure 24R-epimer of (1) are equally suitable, the former providing the 24S-1 α ,25-dihydroxy product and the latter the corresponding 24R-product.



Process Scheme I

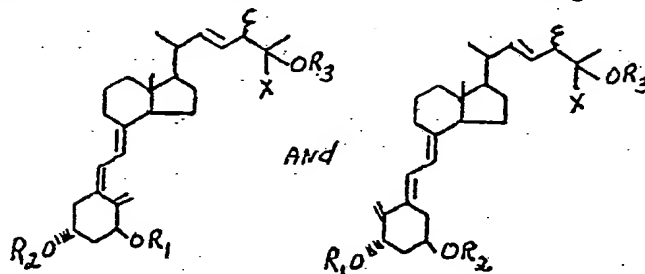


Process Scheme II

Process Scheme III

Claims

1. A compound selected from the group consisting of



wherein each of R_1 , R_2 and R_3 , which may be the same or different, is selected from the group consisting of hydrogen and acyl and X is selected from an alkyl or aryl group or an isotopically labeled alkyl or aryl group, with the proviso that when the C-24-methyl substituent in the 5,6-cis compound has the S-configuration, and X is methyl, R_1 , R_2 and R_3 cannot all be hydrogen.

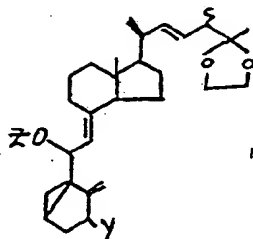
2. The compounds of claim 1 where X is methyl.
3. The compounds of claim 1 where the asymmetric center at C-24 has the (R)-configuration.
4. The compounds of claim 1 where the asymmetric center at C-24 has the (S)-configuration.
5. $1\alpha, 25$ -dihydroxy-24-epivitamin D_2 .
6. $1\alpha, 25$ -dihydroxy-5,6-trans-vitamin D_2 .
7. $1\alpha, 25$ -dihydroxy-5,6-trans-24-epi-vitamin D_2 .
8. A pharmaceutical composition which comprises a compound as claimed in any one of claims 1 to 7 and a pharmaceutically acceptable excipient.
9. A composition according to claim 8, which comprises $1\alpha, 25$ -dihydroxy-5,6-trans-vitamin D_2 and/or $1\alpha, 25$ -dihydroxy-5,6-trans-24-epi-vitamin D_2 .
10. A composition according to claim 8 or 9, which comprises $1\alpha, 25$ -dihydroxy-5,6-trans-vitamin D_2 and $1\alpha, 25$ -dihydroxy-vitamin D_2 .



11. A composition according to claim 8 or 9, which comprises $1\alpha,25$ -dihydroxy-5,6-trans-vitamin D_2 and $1\alpha,25$ -dihydroxy-5,6-trans-24-epivitamin D_2 .
12. A composition according to claim 8 or 9, which comprises $1\alpha,25$ -dihydroxyvitamin D_2 , $1\alpha,25$ -dihydroxy-24-epi-vitamin D_2 , $1\alpha,25$ -dihydroxy-5,6-trans-vitamin D_2 and $1\alpha,25$ -dihydroxy-5,6-trans-24-epi-vitamin D_2 .
13. A composition according to claim 8, which comprises $1\alpha,25$ -dihydroxyvitamin D_2 and $1\alpha,25$ -dihydroxy-24-epi-vitamin D_2 .
14. A pharmaceutical composition which comprises $1\alpha,25$ -dihydroxy-5,6-trans-vitamin D_2 and either $1\alpha,25$ -dihydroxy-5,6-trans-25-epi-vitamin D_2 or $1\alpha,25$ -dihydroxy-24-epi-vitamin D_2 .
15. A composition according to claims 8 or 14 characterized in that it comprises at least one bone mobilization-inducing compound.
16. A composition according to claim 15 wherein the bone mobilization-inducing compound is a vitamin D derivative selected from the group consisting of 25-hydroxyvitamin D_3 , 25-hydroxyvitamin D_2 , 1α -hydroxyvitamin D_3 , 1α -hydroxyvitamin D_2 , $1\alpha,25$ -dihydroxyvitamin D_3 , $1\alpha,25$ -dihydroxyvitamin D_2 , 24,24-difluoro-25-hydroxyvitamin D_3 , 24,24-difluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , 24-fluoro-25-hydroxyvitamin D_3 , 24-fluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , 26,26,26,27,27,27-hexafluoro- $1\alpha,25$ -dihydroxyvitamin D_3 , 26,26,26,27,27,27-hexafluoro-25-hydroxyvitamin D_3 , 2 β -fluoro- 1α -hydroxyvitamin D_3 , 2 β -fluoro-25-hydroxyvitamin D_3 , 24,25-dihydroxyvitamin D_3 , $1\alpha,24,25$ -trihydroxyvitamin D_3 , 25,26-dihydroxyvitamin D_3 , $1\alpha,25,26$ -trihydroxyvitamin D_3 .

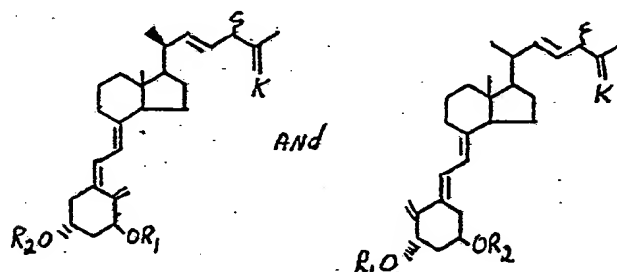


17. Compounds having the formula



wherein Y is hydrogen, hydroxy or O-acyl and Z is an alkyl group.

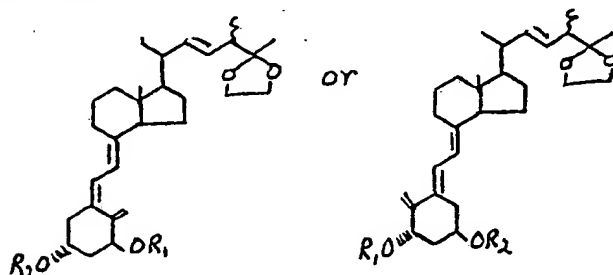
18. The compounds of claim 1 wherein Y is hydrogen.
 19. The compounds of claim 1 wherein Y is hydroxy or O-acetyl.
 20. The compound of claim 2 where Z is methyl.
 21. The compound of claim 3 where Z is methyl.
 22. A compound selected from the group consisting of



where K is an oxygen or ethylenedioxy group, and where R₁ and R₂ which may be the same or different, are hydrogen or acyl.

23. The compounds of claim 5 where K is an oxygen group.
 24. The compounds of claim 5 where K is an ethylenedioxy group.
 25. 1 α -hydroxy-25-oxo-27-nor vitamin D₂ and the acetate thereof.
 26. A process for preparing a compound having a formula as defined in claim 1 wherein each of R₁, R₂ and R₃, which may be the same or different, is hydrogen or acyl and X

is alkyl or aryl or an isotopically labelled alkyl or aryl group which comprises subjecting a ketal of the formula:



wherein R_1 and R_2 are as defined above to hydrolysis at a temperature from 50 to 100°F (10 to 38°C) under acidic conditions and reacting the resulting ketone with a Grignard reagent.

27. A process according to claim 26 wherein the hydrolysis is carried out using p-toluene-sulfonic acid.
28. A method for preventing or treating physiological disorders in mammals, which disorders are characterized by a requirement to regenerate or prevent loss of bone mass, which comprises administering to said mammals a therapeutically effective amount of $1\alpha,25$ -dihydroxy-24-epi vitamin D_2 , alone or in combination with at least one vitamin D compound characterized by its ability to mobilize bone in vivo.
29. The method of claim 1 wherein the disorder is postmenopausal osteoporosis.
30. The method of claim 1 wherein the disorder is senile osteoporosis.
31. The method of claim 1 wherein the disorder is steroid-induced osteoporosis.
32. The method of claim 2 wherein the compound is administered to women during and subsequent to menopause.
33. The method of claim 2 wherein the compound is administered to women prior to the onset of menopause.
34. The method of claim 1 wherein the compound is administer-



ed in an amount from about 0.5 microgram to about 25 micrograms per day.

35. The method of claim 1 wherein the compound, in solution in a liquid vehicle ingestible by and nontoxic to said mammals is administered orally in encapsulated form.
36. The method of claim 1 wherein $1\alpha,25$ -dihydroxy-24-epi vitamin D_2 is the sole compound administered.
37. The method of claim 1 wherein $1\alpha,25$ -dihydroxy-24-epi vitamin D_2 is administered in combination with at least one vitamin D compound characterized by the ability to mobilize bone in vivo.
38. 1α -hydroxy-25-oxo-27-nor-24-epivitamin D_2 .



1/2

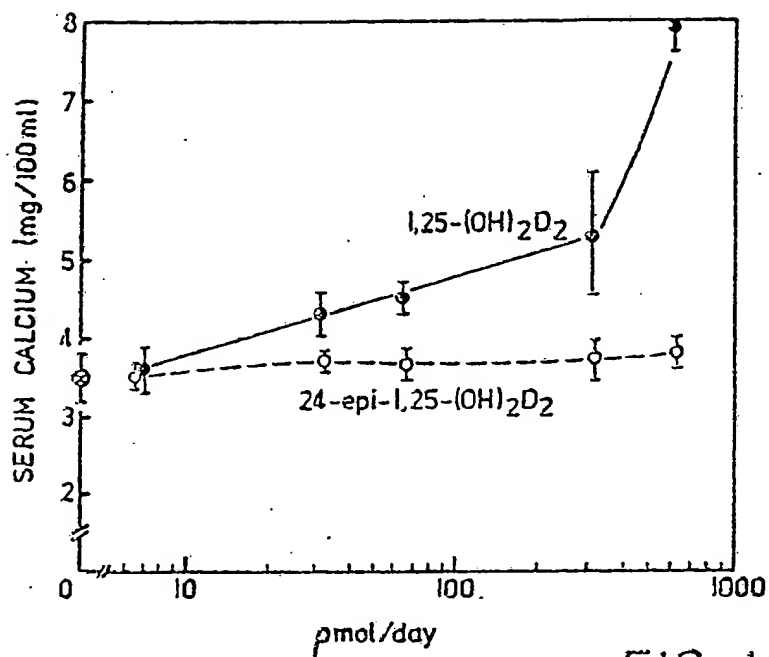


FIG. 1

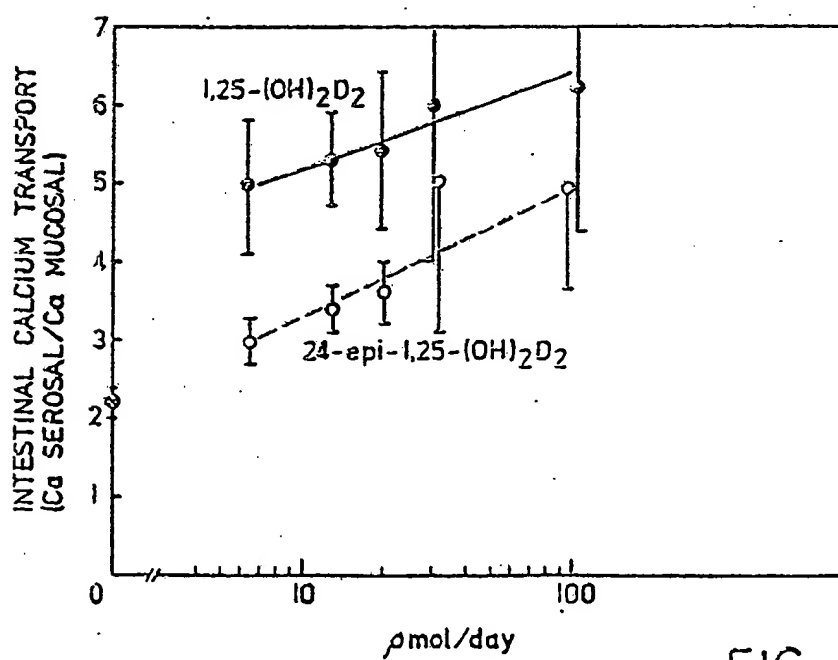


FIG. 2

SUBSTITUTE SHEET



2/2

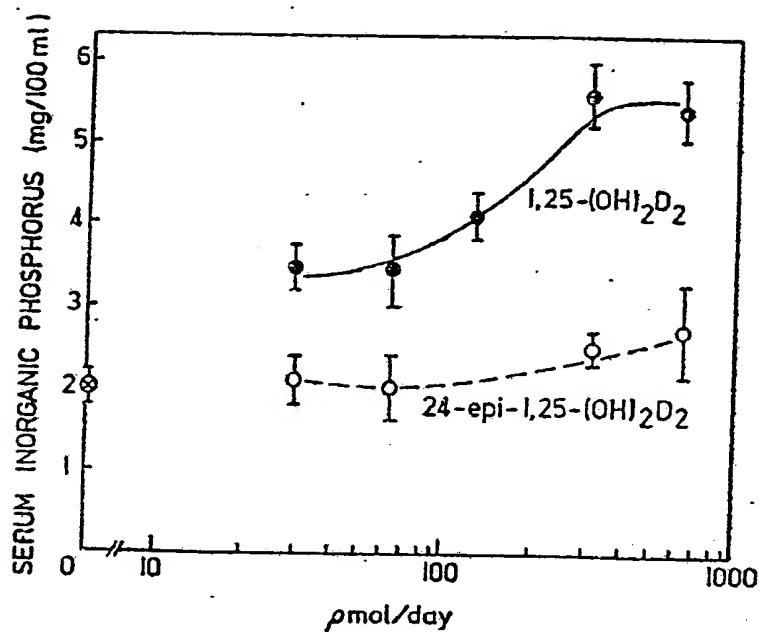


FIG. 3

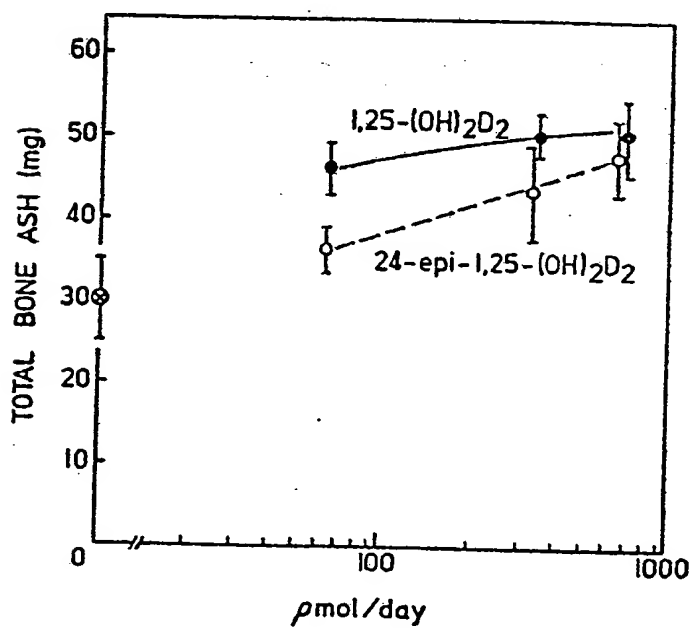


FIG. 4

SUBSTITUTE SHEET



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US84/00714

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. 3 C07J 9/00 U.S. Cl. 260/397.2 A61K 31/59 424/236		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
US	260/397.2 424/236	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
CAS On line Computerized Search Mead Lexis Computerized Search		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	US, A, 4,260,549, published 7 April 1981, De Luca et al. (1)	11-23
A	US, A, 4,267,117, published 12 May 1981, Salmond	
A	US, A, 4,269,777, published 26 May 1981, De Luca et al. (2)	
<p>* Special categories of cited documents: ¹⁶</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ³	Date of Mailing of this International Search Report ³	
3 July 1984	11 JUL 1984	
International Searching Authority ¹	Signature of Authorized Officer ¹⁹	
ISA/US	Elbert Roberts	

